Remarks

Courtesies extended to Applicants' representative at the personal interview held February 19, 2003, are acknowledged with appreciation.

In accordance with the present invention, there are provided transgenic non-human mammals containing at least one FLP recombination target site in their genomic DNA. FLP transgenic mammals may further contain a nucleotide sequence encoding, and capable of expressing a FLP recombinase to effect FLP-mediated recombination. By incorporating a FLP recombination target site, the chromosomal site of transgene integration is controlled, providing a significant advantage over traditional transgenic methodologies that rely on random integration of the transgene. In addition, the level, temporal characteristics, or tissue distribution of transgene expression may be further regulated. For example, specific promoter systems may be used to control FLP recombinase expression, and thus, to control FLP-mediated recombination of a transgene.

Claims 1-19 remain pending in the present application. No amendments have been presented by the present response. The present status of all claims in the application is provided in the listing of claims presented herein beginning on page 2.

The rejection of claims 1-19 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such as a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed, is respectfully traversed. Applicants respectfully submit that the claimed invention is clear and straightforward; and that one of skill in the art, in light of knowledge in the art, together with the teachings of the specification, could readily produce the claimed transgenic mammals.

Applicants respectfully disagree with the Examiner's assertion that the claimed transgenic mammals would require that manipulations with FLP vectors and FLP recombinase take place in animals (see, e.g., Office Action, Paper No. 7, at page 3, lines 1-5). By definition, a

transgenic animal is created upon incorporation of foreign DNA into the genome of an animal. "A eukaryotic organism that develops from a cell into which new DNA has been introduced is called a transgenic organism" (An Introduction to Genetic Analysis, 4th Ed., 1989, Suzuki et al. eds.). In particular, the cells used for the introduction of foreign DNA containing an FLP recombination target site could be embryonic stem (ES) cells, which would then be developed into an organism as is known in the art.

Indeed, as acknowledged by the Examiner, "the specification describes how to introduce said [e.g., FLP] DNA into cells in culture" (see Office Action, Paper No. 7, lines 4-5). Accordingly, one of skill in the art could readily perform the same techniques of DNA introduction into ES cells. These cells would then be developed into an embryo in a surrogate host. Thus, there is no requirement for the introduction of recombinant DNA molecules *in vivo*.

Applicants respectfully submit that one of skill in the art could readily monitor the presence of an FRT recombination target site at a precise location in the genome, even if initially positioned by random integration. For example, the location of the FRT recombination target site could readily be monitored by using PCR analysis to detect the introduced DNA within the genomic DNA. Once the presence of an FLP recombination target site is confirmed, the recombination event promoted by FLP recombinase can then be activated at this precise site, and may be, for example, temporally controlled or activated in only certain cell types as the organism develops from the ES cells.

Therefore, one of skill in the art would have no reason to doubt whether Applicants were in possession of the claimed transgenic animals at the time of filing based on the teaching of the specification. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-19 under 35 U.S.C. § 112, first paragraph.

The rejection of claims 1-19 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention, is respectfully traversed. Applicants respectfully submit that one of skill in the art could readily apply standard ES cell technology

and transgenic techniques in combination with the novel teachings of the specification to make animals containing FLP target site(s) and to use FLP-mediated recombination in these transgenic animals.

Applicants respectfully submit that the invention methods create a recombinant allele or transgenic animal, whether or not any outward phenotype is manifested. In effect, a "molecular phenotype" is defined by the incorporation of foreign introduced DNA containing an FLP recombination target site. It is not necessary to predict the phenotype of the resulting animal before performing the actual modifications of the ES cells, since it is readily determined following introduction/excision of the transgenic DNA of interest. Indeed, the present invention methods provide a novel means of performing recombination to create a transgenic mammal, which is applicable to any gene of interest.

Applicants respectfully submit that the Examiner's comments regarding traditional transgene technologies are inapplicable to the claimed invention (see Office Action, Paper No. 7, at pages 4-6). The goal of using the invention FLP-mediated recombination to produce a transgenic animal was to avoid the very limitations cited. By providing a novel way of specifically controlling the recombination event, the invention provides a means of controlling transgene copy number and timing and location of transgene expression. Thus, the problems of traditional methods of generating transgenics are overcome by the use of controllable FLP-mediated recombination for the introduction and/or excision of DNA within the genome.

Applicants respectfully disagree with the Examiner's assertion that the methods used to create the invention transgenic animal require <u>delivery</u> of recombinase enzyme to all cells (see Office Action, Paper No. 7, at page 6). As discussed above, the introduction of foreign DNA(s) into a transgenic animal can all be performed at the ES cell stage. Thus, if recombination is desired within the ES cells, an FLP protein could be immediately expressed by an expression vector encoding FLP recombinase. Alternatively, if recombination at some later stage of development, or in a particular cell type is desired, an FLP recombinase protein could be expressed at that time or cell through the use of specific promoter sequences. Thus, there is no

need to "deliver the recombinase enzyme to cells of an animal without disrupting recombinase activity by denaturation or degradation", as suggested by the Examiner (see Office Action, Paper No. 7, at page 6). Instead, all that is required is expression of recombinase enzyme to affect recombination as appropriate.

Applicants further disagree with the Examiner's assertion that the methods used to create the invention transgenic animal merely rely on random transgene insertion (see Office Action, Paper No. 7, at pages 7-8). To the contrary, the recombination event is controlled by the action of FLP recombinase on the FLP recombination target site(s) in the genomic DNA. The positioning of the FLP recombination target site construct can be controlled by methods known to one of skill in the art, such as homologous recombination, for example. Subsequent recombination events mediated by FLP recombinase are then specifically targeted to this target site.

Moreover, methods of making and manipulating various mammalian ES cells were known in the art at the time of filing. Indeed, one of the Examiner's own references of record illustrates the state of the prior art, teaching that pluripotent rat, sheep and cattle ES cells capable of producing chimeric offspring have been reported (Mullins and Mullins, *J. Clin. Invest.* 98:S37-S40, 1996).

Therefore, one of skill in the art could readily make and use the transgenic mammals as claimed herein by following the teachings of the specification in light of the knowledge in the art at the time of filing. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-19 under 35 U.S.C. § 112, first paragraph.

The rejection of claims 1-5, and 9-19 under 35 U.S.C. § 103(a), as allegedly being unpatentable over Golic, *Cell* 59:499-509 (hereinafter referred to as "Golic"), 1989 in view of Le Mouellic, *Proc. Natl. Acad. Sci. USA* 87:4712-4716, 1990 (hereinafter referred to as "Le Mouellic") and Rogers WO 87/03006, is respectfully traversed. Applicants respectfully submit that none of these references, either taken alone or in combination, teach or suggest the claimed transgenic mammals containing at least one FLP recombination target site.

Indeed, as acknowledged by the Examiner, Golic does not teach using FLP recombinase to make transgenic animals (see Office Action, Paper No. 7, at page 11). The deficiency of this primary reference cannot be cured by Le Mouellic because Le Mouellic also does not teach or suggest using FLP recombinase to make transgenic mammals. Instead, Le Mouellic teaches the use of homologous recombination, as opposed to recombinase-mediated recombination for the insertion of a transgene. Thus, one of skill in the art would not be motivated to combine the teachings of Golic (FLP-recombinase mediated recombination) with those of Le Mouellic (homologous recombination).

Furthermore, Rogers does not teach or suggest the use of FLP recombinase in a system to produce transgenic mammals. Rogers merely describes manipulation of yeast strains to control gene expression using FLP recombinase.

Therefore, the claimed transgenic mammals could not be rendered obvious by this combination of references. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection under 35 U.S.C. § 103(a).

Conclusion

In view of the above remarks, prompt and favorable action on all claims is respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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